

OVIPOSITION PREFERENCE IN *CERATITIS CAPITATA* (DIPTERA,TEPHRITIDAE): INFLUENCE OF REARING DIET

Iara Sordi Joachim-Bravo¹
Fernando Sérgio Zucoloto¹

ABSTRACT

Experiments were performed to determine the oviposition preference of *Ceratitis capitata* (Wiedemann) in relation to the components of the artificial diet used to raise them in laboratory. The results show that the presence of sucrose and/or citric acid is responsible for making the diets preferred over others that do not contain these substances. The presence of brewer's yeast does not inhibit oviposition, but does not result in preference for a diet unless citric acid and/or sucrose is also present. The flies did not show any preference between sucrose and citric acid. The possibilities of the use of the results obtained are discussed in terms of simplifying the methods for rearing *C. capitata*.

KEYWORDS. *Ceratitis capitata*, fruit fly, oviposition, diets, artificial rearing.

INTRODUCTION

Ceratitis capitata (Wiedemann), the Mediterranean fruit fly, is one of the world's worst agricultural pests, having more than 600 host species most of which are fruit (CHRISTENSON & FOOTE, 1960; BATEMAN, 1972). It is a polyphagous species that responds well to most of its hosts (KRAINACKER *et al.*, 1987; ZUCOLOTO, 1993a). Since this is a species that causes such great harm, one of the pressing concerns of people that study it is how to control its population. In recent years, due to the known dangers of insecticides, techniques of biological

1. Departamento de Biologia; Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo; Av. Bandeirantes 3900; CEP 14040-901 Ribeirão Preto SP; Brazil.

control have gained greater attention (CHEICH *et al.*, 1974; HOOPER, 1987; GARCIA *et al.*, 1989)

The viability of biological control depends on the mass rearing of the fly using artificial diets. Laboratory rearing permits, in addition, the use of the flies in basic research in various areas of biology. Various artificial diets for *C. capitata* have already been developed successfully (MAEDA *et al.*, 1953; PELEG & RHODE, 1970; VARGAS *et al.*, 1983; COCARELI *et al.*, 1986; FAY, 1988). *C. capitata* is reared in the laboratory on a diet based on brewer's yeast. The adults are maintained in cages and the females oviposit through openings in the nylon netting that covers the cage. The eggs fall into a tray with water placed underneath the cage. The eggs are then collected and placed on Petri dishes containing the diet. The same diet is used for larvae and adults.

The main objective was to find out whether the flies oviposit directly over the diets, thus sparing one step in their maintenance process. For this, it would be important to know the nutritive value of the standard diet, as well as how each ingredient influences oviposition behavior.

MATERIAL AND METHODS

The flies used in the experiments are from a rearing system maintained in the laboratory for 14 years (\pm 140 generations), with the flies originating from a wild population obtained from infested peaches of Campinas city (SP) orchards. The original population contained some 5000 wild flies. The standard diet currently used for fly rearing is as follows: distilled water— \rightarrow 80 ml; brewer's yeast (Boneg, Juiz de Fora, Brazil)— \rightarrow 6.5 g; sucrose (União, Rio de Janeiro, Brazil)— \rightarrow 11.0 g; agar-agar (Merck, Darmstadt, Germany)— \rightarrow 3.0 g; citric acid (Merck)— \rightarrow 1.0 g; nipagin in 20% alcohol solution (Merck)— $>$ 1.0 ml; propionic acid (Merck)— \rightarrow 0.5 ml. The diet was manufactured according to ZUCOLOTO *et al.* (1979).

To test the nutritive value of the diets, 10 recently emerged larvae were placed on a Petri dish (90 x 16 mm) lined with lightly moistened filter paper containing 5 g of the diet to be tested. New portions of the diet were added daily until pupation. Each experiment was repeated 6 times. The following parameters were used to test the nutritive value of the diets: percent emergence, time to emergence and wing size (estimated by the distance between the intersection point of $bm-cu$ with CuA_1 and the R_{4+5} apice of the left wing, in 15 females per experiment, fixed previously in 70% alcohol) (ZUCOLOTO, 1987). The data were analyzed statistically by the Kruskal-Wallis test at the 5% level of significance (SIEGEL, 1956). The experiments were carried out in the absence of light, at a temperature of $29 \pm 1^\circ C$ and 70-80% relative humidity.

To test the oviposition preference, 15 pairs of recently emerged flies were placed in acrylic boxes (11 x 11 x 3 cm), and fed a rearing diet and water *ad libitum*. On the eighth day, when the flies reach the peak of oviposition in the laboratory, 2 different types of diets to be tested were introduced into the box. The diets (5 g) were placed on plastic lids (3 cm diameter x 0.5 cm height) and covered with parafilm (American Can Company, Detroit, USA). The parafilm was covered with a piece of red nylon in such a way that the surfaces of the diets had the same color, since we know that color can influence the oviposition preference of the flies (KATSOYANNOS *et al.*, 1986). The eggs laid on each diet were counted after 48 hours. The number of eggs obtained was divided by two in such a way that the results are presented as the number of eggs/female/day. When a female died it was replaced with another in the same physiological state. Each test was repeated 10 times, and the data were analyzed statistically using the Wilcoxon test at the 5% level of significance (SIEGEL, 1956). All of the experiments for oviposition preference were conducted at a temperature of $29 \pm 1^\circ C$ and at a relative humidity of 70-80%, with 10 hours of light per day provided by a 400 lux fluorescent bulb.

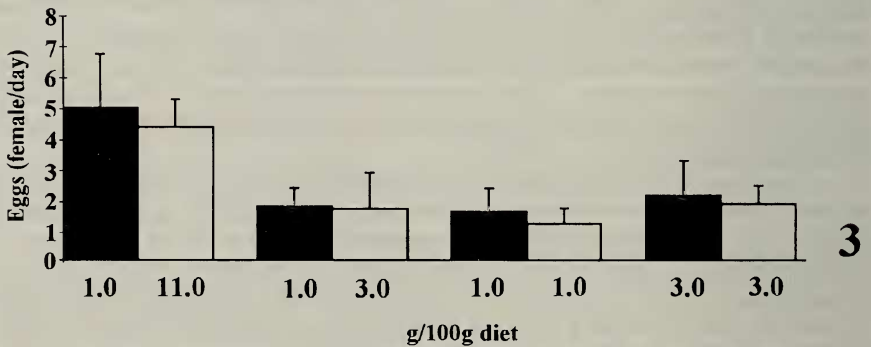
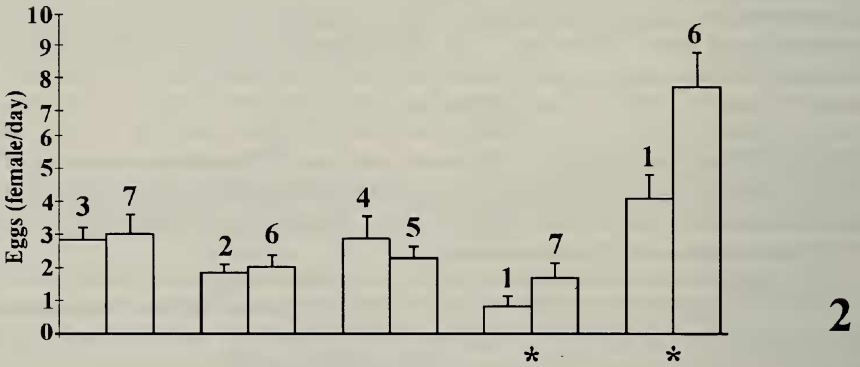
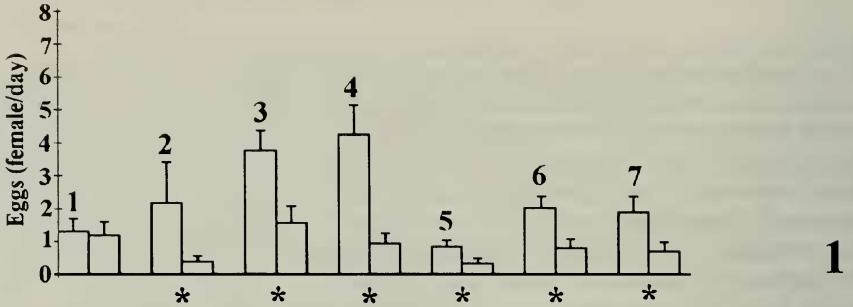
RESULTS

In experiment 1, the objective was to determine the nutritive value of the standard diet used for fly rearing in the laboratory. With this experiment, we would be able to determine if some component of the whole diet could be removed without harming the larvae. Six groups of larvae were reared and fed with the following diets: 1- standard diet; 2- standard diet without citric acid; 3- standard diet without sucrose; 4- diet containing only the yeast; 5- diet containing only sucrose, and 6- diet containing only citric acid. Agar-agar, nipagin and propionic acid were present in all diets. As expected, on diets 5 and 6, the larvae did not survive. The best results were obtained with diet 1, followed by diets 2 and 3, respectively. Diet 4 was slightly inferior to diet 3 (Table I).

In experiment 2, different combinations of the components of the standard diet were tested against agar-agar for female oviposition preference. Nipagin and propionic acid were not tested. The main objective of this experiment was to determine which component(s) of the whole diet would attract fly oviposition to a lesser or greater extent. The following diets were tested against agar-agar: 1- yeast; 2- yeast + citric acid; 3- yeast + sucrose; 4- yeast + sucrose + citric acid; 5- citric acid + sucrose; 6- citric acid and 7- sucrose. The results obtained (fig. 1) indicate that the flies preferentially oviposited on the diets containing citric acid and sucrose, even those that contain yeast. There was no preference when the yeast was tested alone against agar-agar.

In experiment 3, oviposition preference was compared between diets with nutritive value adequate for larvae, i.e., containing yeast, against diets containing citric acid and sucrose where the larvae did not develop. In this experiment, the objective was to find out if there would be a relationship between the oviposition preference of the females and the diets that provide the larvae with better performance. The following diets were tested: 1- yeast + sucrose vs. sucrose; 2- yeast + citric acid vs. citric acid; 3- yeast + citric acid + sucrose vs. citric acid + sucrose; 4- yeast vs. citric acid, and 5- yeast vs. sucrose. The results (fig. 2) indicate that there was no relationship between the diets preferred for oviposition and better larval development. These results also indicate that citric acid and sucrose are the determinant factors in female oviposition choice, confirming the results of the earlier experiment.

Based on the results obtained in experiments 2 and 3, others were conducted to see if there would be female oviposition preference between citric acid and sucrose. The following tests were carried out: 1- citric acid 1.0 g vs. sucrose 11.0 g (same quantities as used in the rearing diet); 2- citric acid 1.0 g vs. sucrose 3.0 g (quantities detected in some of the fruit used for this species, according to WHITING, 1970); 3 and 4 - citric acid and sucrose tested in equal quantities, 1.0 and 3.0 g, respectively. The results obtained (fig. 3), show there was no preference for citric acid or sucrose in either the experiments.



Figs. 1-3. Oviposition preference by *Ceratitis capitata*: 1, When offered different diets against an agar diet; 2, When offered different diets with and without yeast; 3, When offered only citric acid (dark columns) against only sucrose (light columns). The results are the mean (\pm SD) of ten replicates. (*) There is statistical differences (Wilcoxon, 5%). 1=yeast; 2=yeast+citric acid; 3=yeast+sucrose; 4=yeast+citric acid+sucrose; 5=citric acid+sucrose; 6=citric acid; 7=sucrose; without number=agar.

Table I. Nutritional value of yeast diets for laboratory *Ceratitis capitata* larvae. The results show the means (\pm SD) of six replications. Means, within columns, followed by different letters differ from one another ($p < 0,05$, Kruskal-Wallis test). Y-yeast, S-sucrose, CA - citric acid. With sucrose and citric acid diets, no larvae survived.* Wing size estimated by the distance between the intersection point of $bmcu$ with CuA_1 and the R_{4+5} apice of the left wing.

Diets	Emergence (%)	Time to emergence (days)	Wing size* (mm)
Y+S+CA	77.00 \pm 7.57 ^a	16.57 \pm 0.19 ^a	2.89 \pm 0.04 ^a
Y+S	68.00 \pm 3.46 ^{ab}	17.42 \pm 0.34 ^{ab}	2.84 \pm 0.10 ^a
Y+CA	41.00 \pm 12.05 ^{ab}	17.46 \pm 0.15 ^{ab}	2.57 \pm 0.15 ^b
Y	34.00 \pm 4.00 ^b	17.93 \pm 0.21 ^b	2.69 \pm 0.13 ^b

DISCUSSION

The results obtained in the experiments indicate: (1) Removal of citric acid and/or sucrose from the standard diet hinders larval development; (2) there is no correlation between the nutritive value of the diets for the larvae and the choice by females to oviposit preferentially on these diets; (3) citric acid and sucrose are the substances that determine female oviposition preference in the diets; (4) yeast, when offered separately in the diet, is not preferred by females for oviposition; (5) brewer's yeast, when in the diet together with citric acid and/or sucrose, does not inhibit oviposition, indicating that it is not a deterrent.

The great variation in the quantity of ovules from one experiment to another was probably due to diet utilization by flies that emerged at different times. Although the rearing method is uniform throughout the year, the nutritional component of brewer's yeast may vary depending on the lot used (CANGUSSU & ZUCOLOTO, 1992, 1995).

The standard diet - containing yeast, citric acid and sucrose - provided the best results for larval development. Probably, this is due to the following: generally, for adequate development insects need a source of amino acids or proteins, a source of carbohydrates, generally sugars, B complex vitamins, some salts, and, depending on the species, vitamins A and C, and lipids (DADD, 1985). Brewer's yeast satisfies the amino acid, B complex vitamin and salt requirements (VANDERZANT, 1974; FRANCO, 1982) and sucrose satisfies the sugar requirements, in addition to being a phagostimulant (HSIAO, 1985). In addition, the presence of citric acid may also be a phagostimulant for larvae (ZUCOLOTO, 1991) and may maintain an adequate pH for larval development (FAY, 1988).

The removal of citric acid or of sucrose caused a lowering of larval performance, with the removal of sucrose being more harmful than that of citric acid. This fact perhaps is due to the functions of sucrose, which are to provide energy and to act as a phagostimulant (HSIAO, 1985; DADD, 1985). With the removal of sucrose, all the energy for the maintenance of the larvae would come

from the brewer's yeast, although citric acid may play this role (GILMOUR, 1968). However, the quantity of citric acid in the diet (1.0 g) would not be capable replacing sucrose *in toto* in this function. As known, in the absence of carbohydrates, amino acids shift their structural function to energy, which can have a harmful effect on larval performance (DADD, 1985). With the removal of citric acid and sucrose, the structural, energy, and phagostimulant functions may be taken on by yeast. Since yeast is not a phagostimulant for the larvae (ZUCOLOTO, 1987, 1991), the larvae may reduce ingestion of the diet, hindering their development. However, it should be pointed out that with the diet containing only yeast, 34% of the larvae reached the adult stage, which may indicate variability in the population in relation to the phagostimulant action of the yeast and/or in relation to the efficient transformation of amino acids into an energy source. Similar results were obtained when one diet, also based on brewer's yeast, was tested for this population (ZUCOLOTO, 1987).

Citric acid and especially sucrose are components of a large number of hosts used by *C. capitata* (WHITING, 1970). Perhaps this fact explains why these two substances attract the flies to oviposit on the diets that contain them. Sucrose can even signal that a fruit is ripe. It is known that females of *C. capitata* prefer to oviposit on ripe fruit (OI & MAU, 1989). The lack of fly preference between citric acid and sucrose reinforces our previous statement, i.e., the two substances act as signals for the presence of hosts. The responses of some species of Tephritidae to the stimulation of carbohydrates, in terms of feeding and oviposition, are variable. Glucose and fructose stimulate oviposition in *Rhagoletis pomonella* (Walsh) and *R. completa* (Cresson) (TSIROPOULOS & HAGEN, 1979). For *Dacus tryoni* (Froggatt), glucose and sucrose do not stimulate oviposition, but fructose does (EISEMANN & RICE, 1985). Sucrose stimulates oviposition in *Anastrepha suspensa* (Loew) (SZENTESI *et al.*, 1979).

Although yeast may not be preferred by the flies, when offered without citric acid or sucrose, its presence in the diets does not inhibit oviposition, which may indicate that the yeast is not a deterrent for *C. capitata*. Probably the yeast lacks attractive substances for this species. It is interesting to point out that this population, although raised for 15 years on a diet based on brewer's yeast, was not selected to prefer it in terms of oviposition behavior. Furthermore, the population seems to still prefer substances found in natural hosts, indicating strong genetic control over this behavior. We want to point out that only recently (1995) we introduce citric acid and sucrose (in place of honey) into the rearing diet. Therefore, it would have been difficult for selection to occur in the laboratory for the preference of oviposition on diets containing citric acid or sucrose.

The lack of preference for oviposition on the diet containing only yeast in relation to the diets containing citric acid and/or sucrose shows that females do not select the best diets for the immature for oviposition. A theoretical discussion of the oviposition/performance relationship based on our reported data is not possible using flies raised on artificial diets in the laboratory, as was the case for the present experiments. However, in other experiments where wild flies and natural hosts were utilized, it was shown that this correlation was also absent (Joachim-Bravo & Zucoloto, unpublished). Perhaps the polyphagous behavior of the species,

frequently associated with the unpredictability of resources, explains this lack of correlation (KRAINACKER *et al.*, 1987).

In conclusion, we can state that at least for small rearing operations, the results may be applied with the aim of simplifying the rearing of *C. capitata*. Preliminary results show that although a certain portion of the eggs are laid on the walls of the boxes, a sufficient amount to maintain the rearing is oviposited on the diet. We do not know if this method would work for mass rearing. A possible suggestion is to select females that oviposit preferentially on the diet and not on the walls of the box. Earlier results have shown that, even in populations of *C. capitata* raised for a long period of time in the laboratory, there exists sufficient variability for the selection of some characteristics (BOLLER & CALKINS, 1984; LEMOS *et al.*, 1992; ZUCOLOTO, 1993b; CANATO & ZUCOLOTO, 1993).

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